REVIEW

Effects of Probiotics on Necrotizing Enterocolitis, Sepsis, Intraventricular Hemorrhage, Mortality, Length of Hospital Stay, and Weight Gain in Very Preterm Infants: A Meta-Analysis

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ABSTRACT

Probiotics are increasingly used as a supplement to prevent adverse health outcomes in preterm infants. We conducted a systematic review, meta-analysis, and subgroup analysis of findings from randomized controlled trials (RCTs) to assess the magnitude of the effect of the probiotics on health outcomes among very-low–birth-weight (VLBW) infants. Relevant articles from January 2003 to June 2017 were selected from a broad range of databases, including Medline, PubMed, Scopus, and Embase. Studies were included if they used an RCT design, involved a VLBW infant (birthweight <1500 g or gestational age <32 wk) population, included a probiotic intervention group, measured necrotizing enterocolitis (NEC) as a primary outcome, and measured sepsis, mortality, length of hospital stay, weight gain, and intraventricular hemorrhage (IVH) as additional outcomes. The initial database search yielded 132 potentially relevant articles and 32 (n = 8998 infants) RCTs were included in the final meta-analysis. Subgroup analysis was used to evaluate the effects of the moderators on the outcome variables. In the probiotics group, it was found that NEC was reduced by 37% (95% CI: 0.51%, 0.78%), sepsis by 37% (95% CI: 0.72%, 0.97%), mortality by 20% (95% CI: 0.67%, 0.95%), and length of hospital stay by 3.77 d (95% CI: −5.94, −1.60 d). These findings were all significant when compared with the control group. There was inconsistent use of strain types among some of the studies. The results indicate that probiotic consumption can significantly reduce the risk of developing medical complications associated with NEC and sepsis, reduce mortality and length of hospital stay, and promote weight gain in VLBW infants. Probiotics are more effective when taken in breast milk and formula form, consumed for <6 wk, administered with a dosage of $<10^9$ CFU/d, and include multiple strains. Probiotics are not effective in reducing the incidence of IVH in VLBW infants. *Adv Nutr* 2017;8:749–63.

Keywords: very preterm infants, probiotics, necrotizing enterocolitis, sepsis, mortality, hospital admission

Introduction

Survival rates of very preterm infants [very-low–birth-weight (VLBW) infants born <32 wk gestation or with a birth weight of <1500 g)] are now >90% thanks to advances in medical technology. However, with decreasing gestational age and birthweight, there is a concomitant increasing risk of short-term and long-term developmental complications and problems (1), which may be associated with mortality and poor neurodevelopmental outcomes (2).

Among the complications, necrotizing enterocolitis (NEC) is one of the most serious conditions in VLBW infants (3) and is a leading cause of death in this population (4). Infants diagnosed with NEC have a higher chance of developing poor brain growth and associated neurodevelopmental delays (2). Although the pathogenesis of NEC is multifactorial and remains unclear, it is thought that because the gut microbiota of VLBW infants may be very immature, exposure to pathogenic organisms would cause mucosal injury related–inflammatory response, leading to NEC (5).

Late-onset sepsis is another common cause of death in VLBW infants, with ∼20% of all deaths attributable to this condition (6). VLBW infants are diagnosed with sepsis

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Supplemental Figures 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://advances.nutrition.org.

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Abbreviations used: IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; RCT, randomized controlled trial; VLBW, very low birth weight.

when ≥1 blood culture obtained after 72 h of life are identified as positive for pathogenic bacteria (6). Sepsis is associated with increased mortality, morbidity, and prolonged hospitalization (6).

Intraventricular hemorrhage (IVH) is a condition found predominantly in preterm infants in whom fluctuation of blood pressure and impaired cerebrovascular regulation leads to bleeding into the ventricular system of the brain (7). As a result of IVH, VLBW infants have been found to have longer stays in the hospital than full-term infants (8), making them more vulnerable to nosocomial infections.

Newborns, especially VLBW infants, have not yet established a microecological balance of the intact intestinal barrier and therefore have immature immune function. Consequently, they have low resistance to infection. Pathogenic bacteria can pass through the intestinal mucosa more easily and enter the tissues of the mesenteric lymph nodes, organs, and blood flow, causing intestinal infections, such as diarrhea, neonatal necrotizing enterocolitis, and sepsis, all of which are life threatening. VLBW infants (birth weight <1500 g) develop a very different, "sparse" microbiome than that described for the full-term infant (9). The immature and fragile gut in VLBW infants, the in utero environment (e.g., prenatal maternal illness, infections, smoking, and physiological stress), and postnatal factors, such as formula feeding, invasive procedures, antibiotics, and medications, can all alter the gastroenteric pH level. In addition, VLBW infants often require prolonged hospitalization, which may affect their resistance and gastrointestinal microbiota, thereby increasing the risk of infection and inflammation (10).

In recent years, there has been increased interest in the use of probiotics in VLBW infants for the promotion of growth and development and prevention of various complications. Probiotics are defined as "live microorganisms [that], when consumed in adequate amounts, confer a health effect on the host" (11). The supplementation of probiotics may improve gastrointestinal tolerance (12). The possible mechanism through which probiotics reduce the risk of VLBW infants developing NEC is by colonizing their gastrointestinal tract with normal flora. This prevents overgrowth of pathogenic bacteria and reduces the risk of NEC, sepsis, and IVH (13), leading to a reduction in mortality in VLBW infants (14).

RCTs examining the effectiveness of probiotics on growth in VLBW infants have demonstrated mixed results. A few trials have shown improved growth (5, 15), some trials have shown significant differences between treatment and control groups in NEC (16), sepsis (17), subsequent mortality (16), and shortened length of hospital stay (18), whereas others have found no differences (19, 20). Although there are published meta-analyses that include a small number of articles (5, 15, 19, 20) or focus on infants <37 wk of gestation (16), there are no meta-analyses that examine the most recent evidence in VLBW infants born with a birth weight <1500 g or who are born at 32 wk of gestation. The present study aimed to conduct a meta-analysis of randomized control trials published in the last 14 y that used a large sample size to evaluate if probiotics have beneficial effects in VLBW infants, particularly in relation to reduction of NEC. In addition, this study assessed important health outcomes in VLBW infants, including medical complications, such as sepsis and IVH, along with mortality, prolonged length of hospital stay, and weight gain.

Methods

Search methods. The review protocol was registered with the Prospero International Prospective Register of Systematic Reviews (http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID = CRD42016036557; registration number CRD42016036557 (21). The Population, Intervention, Control, Outcome principle was used to search the literature (21). The population was VLBW infants born <32 wk of gestation and weighing <1500 g, the intervention was the probiotic supplementation to the intervention group, the control was a control or placebo group that did not receive probiotic supplementation, and the outcomes included NEC, sepsis, IVH, neurodevelopmental growth involving weight gain, length of hospital stay, and mortality.

The keywords used to identify relevant studies were as follows: very preterm infants and probiotics and randomized controlled trial; very preterm infants and probiotics and growth and randomized controlled trial; and very preterm infants and probiotics and infections and randomized controlled trial. For cases where data were not adequately reported, authors were contacted in an attempt to retrieve the necessary information.

Inclusion criteria. The criteria used to determine inclusion of studies in the meta-analysis were as follows: *1*) publication in peer-reviewed literature in the last 14 y (January 2003 until June 2017); *2*) study design involved a RCT; *3*) population included VLBW infants with gestational age <32 wk or birth weight <1500 g; *4*) probiotics were used as the intervention approach; *5*) outcome variables included NEC as the primary outcome and sepsis, mortality, IVH, length of hospital stay, and weight gain as additional outcomes; and *6*) if multiple studies were published on the same population, the most recent one was included in the analysis.

Data collection. For articles with insufficient data reported, the corresponding authors were contacted to seek further information and acquire the necessary data. Thirteen authors were contacted for weight at the beginning and end of the study (12, 14, 20, 22–31). Some authors replied with the weight gain (24, 27, 31), and their values were included in the analysis.

Data extraction. The process of identifying relevant articles for inclusion in the meta-analysis is described in **Figure 1**. Relevant articles were selected from a broad range of databases, including PubMed, Scopus, and Embase. The following data were extracted from each of the included articles: study setting, participant demographics, study population, type of probiotics used in the intervention, type of control used, the details of primary and secondary end points, study methodology, blinding, study completion rates, times of measurement, indicators of acceptability to users, suggested mechanisms of intervention action, information for assessment of the risk of bias and reported major findings.

Quality assessment. The Physiotherapy Evidence Database tool was used to assess the quality of the articles (32). This tool categorizes the quality of studies into 3 levels: high quality (≥8 points), moderate quality (4–7 points), and low quality (≤3 points). These levels are based on criteria as follows: random allocation of subjects, concealed randomization, similarity of baseline information between groups, blinding to subjects, assessors, and researchers, attrition rate, maintenance of group allocations, use of intention-to-treat analysis, use of variability measures, and use of between-group comparison methods.

Statistical analysis. The effect of probiotics on health outcome variables was described with the use of a pooled effect size method. Random-effect meta-analyses were performed to describe the overall effect size. Risk ratio was used

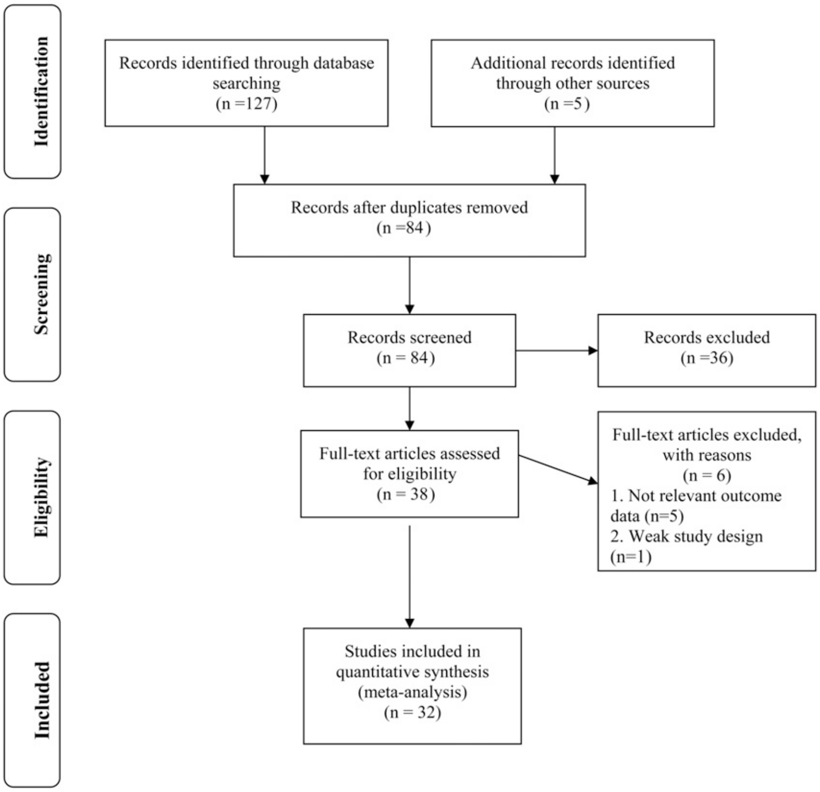

FIGURE 1 The Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow chart representing the reviewing process.

to present the effect size for NEC, sepsis, mortality, and IVH, and pooled mean differences for length of hospital stay and weight gain were calculated. CIs and *P* values were calculated for each of the outcome variables. Several parameters were used to assess the inconsistency between the pooled studies. These included visual inspection of CI overlap and I^2 statistics. Subgroup analyses were performed, including quality of studies (high compared with moderate), dosage of probiotics ($\geq 10^9$ CFU compared with $<10^9$ CFU), strains of probiotics (single compared with multiple strains), and the forms of probiotics used (milk compared with formula or a combination of breast milk and formula). The duration of probiotics intervention ($\geq$6 wk compared with <6 wk), the effect of probiotics on gestational age ($\geq$29 wk compared with <29 gestation wk), and on infant birth weight ($\leq$1000 g compared with >1000 g) were also analyzed. Publication bias was assessed with the use of the Egger test, and funnel plots were presented. Sensitivity analyses were conducted to estimate the effect of probiotics owing to a particular study or studies.

Results

The initial database search yielded 127 potentially relevant articles, and 5 additional articles were found through the reference lists of published articles. Duplicate articles (84) were removed. A total of 43 studies remained, and their titles and abstracts were screened to find any relevant articles for the topic. Following application of the Physiotherapy Evidence Database process, 38 articles remained. Of these, 5 articles were excluded due to lack of relevant outcome data (n = 4) (33–36) and use of a nonrandomized controlled trial design (n = 1) (37). This resulted in 32 studies being included in the final meta-analysis.

Participants. Full details of the included studies are provided in **Table 1**. The 32 included studies reported outcomes on 4,496 infants treated with probiotics and 4,452 infants in control groups. The third inclusion criterion for all the studies was a population consisting of infants with either a birth weight <1500 g or gestational age <32 wk. Six studies met this criterion based on gestational age (14, 26, 29, 38, 39, 45, 51), 8 based on birth weight (3, 5, 20, 40–42, 44, 47), and the remaining studies included both gestational age and birth weight (12, 14, 19, 22–25, 27, 28, 30, 31, 46, 48–50, 52–54).

Probiotics were initiated at different times, ranging from the first feed to 7 d after birth. Fifteen studies introduced probiotics or placebo with the first feeding (5, 12, 19, 22, 27, 28, 31, 39–42, 46, 48–50, 52), 5 studies initiated probiotics on the second day of birth (3, 20, 38, 47, 51) and 2 trials initiated probiotics within 3 d of life (30, 44), within a week (24, 29), and after the first week (14, 24, 53, 54). Only 1 study introduced probiotics after the initiation of breast milk (45), from day 4 of life (26), or when a VLBW infant was receiving 1 mL of breast milk every 4 h (25). The studies were conducted in 19 different locations, including Turkey (22, 23, 28, 46, 50), France (24, 48), Germany (26, 45), Greece (29, 51), India (30, 49), Bangladesh (52), Taiwan (19, 42), Japan (20, 40), Brazil (3), China (14), Colombia (47), England (38), Italy (44, 53, 54), Israel (5), Mexico (39), Slovenia (41), South Africa (31), Australia (27), or in multiple centers (i.e., Australia and New Zealand) (25), and the United States (12).

Effects on NEC. Twenty-five studies with a total sample of 8492 infants measured rates of infant NEC. The mean NEC

TABLE 1 Characteristics of 29 included randomized controlled trials[1]

Study (year) (ref.)	Participants at beginning, *n* (P/C)	Design; location	Gestational age, P/C, wk	Sex, *n* P/C, M/F	Birth weight, P/C, g	Probiotic route of administration; duration, P/C	Probiotics strain(s)	Dose, CFU/g	Form of probiotics	Placebo group	Measured outcomes and results	Attrition, *n* (%)	Key results
Al-Hosni et al. (2012) (12)	101 (50/51)	DB; United States	25.7 (1.4)/25.7 (1.4)	22/28; 28/22	778 (138)/779 (126)	Enteral; 28 d	*Lactobacillus rhamnosus* GG, *Bifidobacterium infantis*	5×10^8	Breast milk	Breast milk	Weight gain, CLD, ROP, NEC, sepsis, mortality, mean volume of feeding	0 (0)	501–750-g infants had significant weight gain
Bin-Nun et al. (2005) (5)	145 (72/73)	SB; Israel	29.8 (2.6)/29.3 (4.3)	44/28; 37/36	1152 (262)/ 1111 (278)	Oral; 36 wk PMA	*B. infantis, Streptococcus thermophilus, B. bifidus.*	1.05×10^9	Breast milk and preterm formula	Breast milk or formula	Weight gain, NEC, TPN	0 (0)	NEC rate was reduced in probiotics group (4% vs. 16.4% in the control group; *P* = 0.03). NEC was less severe in the probiotics group
Braga et al. (2011) (3)	231 (119/112)	DB; Brazil	29.5 (2.5)/29.2 (2.6)	58/61; 55/57	1194.7 (206.3)/ 1151.4 (224.9)	Oral; 28 d	*B. breve, L. casei*	3.5×10^7 3.5×10^9	Breast milk	Breast milk	NEC, sepsis, death	12 (4.93)	4 confirmed NEC cases; ≥2 occurred only in control group
Chou et al. (2010) (14)	301 (153/148)	SB; China	28.5 (2.3)/28.5 (2.3)	83/70; 85/63	1103.6 (232.4)/ 1097 (231.4)	Oral; until discharge	*L. acidophilus, B. infantis*	10^9 CFU for each strain	Breast milk	Breast milk	Growth: height, weight, HC, neurodevelopmental and sensory; outcomes: NEC, death, CLD, sepsis, hospitalization, visual impairment, deafness, cerebral palsy	0 (0)	Mortality was less in probiotics group compared with control group (8 cases, 5.2% vs. 24 cases, 16.2%; *P* = 0.002)
Costalos et al. (2003) (29)	87 (51/36)	SB; Greece	Not mentioned	24/27; 23/13	Not mentioned	Enteral; 30 d	*Saccharomyces boulardii*	10^9	Preterm formula	Maltodextrins with preterm formula	Weight gain and loss, daily milk intake, NEC, sepsis, bacterial counts	0 (0)	No difference in weight gain, NEC, and sepsis
Costeloe et al. (2016) (38)	1310 (650/660)	DB; England	27.88 (1.65)/27.9 (1.75)	374/276; 370/290	1039 (312)/ 1043 (317)	Oral; 36 wk PMA or discharge	*B. breve* BBG-001	10^9	Breast milk	Corn starch with breast milk	ROP, death, NEC, sepsis	5 (0.38)	No effects on death, NEC and sepsis
Demirel et al. (2013) (22)	271 (135/136)	DB; Turkey	29.4 (2.3)/29.2 (2.5)	69/66; 66/70	1164 (261)/ 1131 (284)	Oral; until discharge	*S. boulardii*	5×10^9	Breast milk and preterm formula	Breast milk or formula	Feeding amount, full feeding day, weight, NEC, sepsis	7 (2.5)	Feeding intolerance and sepsis are lower in the probiotics group
Dilli et al. (2015) (23)	200 (100/100)	DB; Turkey	28.8 (1.9)/28.2 (2.2)	53/47; 58/42	1236 (212)/ 1147 (271)	Oral; NICU stay 34.5 (11)/37 (18)	*B. lactis*	5×10^9	Breast milk and preterm formula	Breast milk or formula + maltodextrin	Height, weight, HC, NEC, sepsis, feeding intolerance, RDS, stay at NICU, mortality	0 (0)	NEC rate was lower in probiotics group (2% vs. 18% in placebo group). Sepsis and mortality rate were lower, and hospital stay was shorter in the probiotics group
Fernandez et al. (2013) (39)	150 (75/75)	DB; Mexico	30.95 (4.7)/31.25 (4.5)	Not mentioned	Not mentioned	Oral or enteral; 51.75 (57.5)/47.25 (55.5)	*L acidophilus, L. rhamnosus, L. casei, L. plantarum, B. infantis, S. thermophillus*	1.0×10^9 4.4×10^8 1.0×10^9 1.76×10^8 2.76×10^7 6.6×10^5	Breast milk and preterm formula	Breast milk or preterm formula	Weight, death, NEC	0 (0)	NEC was reduced
Hays et al. (2016) (24)	102 (50/52)	DB; France	Not mentioned	Not mentioned	Not mentioned	Oral; 4–6 wk	*B. lactis*	10^9	Maltodextrin + water	Maltodextrin with sterile water	Weight, length, HC, NEC, sepsis, body composition, bacterial count	22 (21.6)	No differences were found in all outcome variables

(Continued)

TABLE 1 (*Continued*)

Study (year) (ref.)	Participants at beginning, *n* (P/C)	Design; location	Gestational age, P/C, wk	Sex, *n* P/C, M/F	Birth weight, P/C, g	Probiotic route of administration; duration, P/C	Probiotics strain(s)	Dose, CFU/g	Form of probiotics	Placebo group	Measured outcomes and results	Attrition, *n* (%)	Key results
Hikaru et al. (2010) (40)	208 (108/100)	SB; Japan	28.5 (2.6)/28.1 (2.8)	58/50; 44/56	1066 (272)/ 1009 (267)	Oral; until discharge	*B. breve*	10^9	Breast milk and preterm formula	Breast milk or preterm formula	Hospitalization, sepsis, death	0 (0)	Sepsis was lower in the probiotics group. Weight gain was greater and mortality rate was lower in the probiotics group
Jacobs et al. (2013) (25)	1099 (548/551)	DB; Australia and New Zealand	27.9 (2.0)/27.8 (2.0)	272/276; 300/248	1063 (259)/ 1048 (260)	Oral; until discharge or term-corrected age	*B. infantis*, *S. thermophilus*, *B. lactis*	3×10^8, 3.5×10^8, 3.5×10^8	Breast milk and preterm formula with maltodextrin	Maltodextrin	Sepsis, death, hospitalization, duration of parenteral nutrition, days to full enteral feeds, weight at 28 d, ROP, CLD, IVH	0 (0)	Reduced NEC rate
Kanic et al. (2015) (41)	80 (40/40)	SB; Slovenia	28.25 (0.75)/28.55 (0.95)	22/18; 27/13	1104.1 (233.2)/ 1024.3 (249.9)	Enteral; until discharge	*L. acidophilus*, *Enterococcus faecium*, *B. infantum*	1.2×10^7	Breast milk	Breast milk or formula	Hospitalization, late-onset sepsis, pneumonia, NEC, death, meningitis, urinary tract infection, and omphalitis	0 (0)	Lower incidence of sepsis in probiotics group than in control group ($P = 0.002$)
Lin et al. (2005) (42)	367 (180/187)	SB; Taiwan	28.5 (2.5)/28.2 (2.5)	84/96; 100/87	1104 (242)/ 1071 (243)	Oral or enteral; until discharge	*L. acidophilus*, *B. infantis*	10^6 of each	Breast milk	Breast milk	Hospitalization, death, NEC, sepsis	0 (0)	Death and NEC were lower in probiotics group than in control group
Lin et al. (2008) (43)	434 (217/217)	SB; Taiwan	Not mentioned	122/95; 115/102	1028.9 (246)/ 1077.3 (214.4)	Oral; 6 wk	*L. acidophilus*, *B. bifidum*	10^9	Breast milk and preterm formula	Breast milk or formula	NEC, sepsis, death, IVH, CLD, NICU stay, weight gain	9 (2)	Death ($P = 0.002$) and NEC ($P = 0.02$) were lower in probiotics group than in control group
Manzoni et al. (2006) (44)	80 (39/41)	DB; Italy	29.5 (5)/29.3 (4)	20/19; 21/20	1212 (290)/ 1174 (340)	Oral; 6 wk or until discharge	*L. rhamnosus*	6×10^9	Breast milk	Breast milk	Hospitalization, time of achievement of full feedings, death, NEC	0 (0)	NEC was slightly lower in the probiotics group ($P = 0.53$)
Mihatsch et al. (2010) (45)	180 (91/89)	SB; Germany	26.6 (1.8)/26.7 (1.7)	55/36; 47/42	856 (251)/871 (287)	Oral; 6 wk	*B. lactis*	6×10^9	Breast milk and preterm formula	Breast milk or preterm formula	NEC, death, nosocomial infections	17 (9.3)	All variables were not statistically significant
Mohan et al. (2008) (26)	69 (37/32)	DB; Germany	31.05(2.31)/31.27 (2.56)	14/23; 11/21	1449 (343)/ 1398 (331)	Oral; 21 d	*B. lactis* Bb12	2×10^9	Sterile water	Human milk fortifier without probiotics: Nestlé FM 2000B	Body weight, alteration of gut fermentation patterns, and immunologic parameters	0 (0)	Higher body weight in the probiotics group than in placebo group ($P = 0.002$)
Oncel et al. (2014) (46)	400 (200/200)	DB; Turkey	28.2 (2.4)/27.9 (2.5)	108/92; 98/102	1071 (274)/ 1048 (298)	Oral; until discharge	*L. reuteri*	1×10^8	Oil base	Oil base	NEC, sepsis, death, hospitalization, feeding intolerance	24 (6)	Sepsis and duration of hospital stay were lower in probiotics group than in placebo group
Patole et al. (2014) (27)	153 (77/76)	DB; Australia	28.33 (2.96)/27.67 (2.22)	45/320; 41/35	1041.67 (388.89); 1031.67 (333.33)	Oral; until 37 wk PMA	*B. breve* M-16V	3×10^9	Breast milk or water	Dextrin with breast milk or sterile water	Discharge weight, death, hospitalization	6 (3.8)	No difference between probiotics and control group in all variables

(*Continued*)

TABLE 1 (*Continued*)

Study (year) (ref.)	Participants at beginning, *n* (P/C)	Design; location	Gestational age, P/C, wk	Sex, *n* P/C, M/F	Birth weight, P/C, g	Probiotic route of administration; duration, P/C	Probiotics strain(s)	Dose, CFU/g	Form of probiotics	Placebo group	Measured outcomes and results	Attrition, *n* (%)	Key results
Rojas et al. (2012) (47)	750 (372/378)	DB; Colombia	31.75 (1.5)/31.5 (2)	186/186; 185/193	1515.75 (124.25)/ 1477.75 (155.25)	Oral or enteral; until discharge	*L. reuteri*	10^8	Oil base	Oil-based suspension	Death, duration of hospital, NEC, sepsis	0 (0)	Duration of hospitalization decreased in premature infants ≤1500 g (*P* = 0.03)
Rougé et al. (2009) (48)	94 (45/49)	DB; France	28.1 (1.9)/28.1 (1.8)	28/17; 26/23	1115 (251)/ 1057 (260)	Oral; until discharge	*L. rhamnosus*, *B. longum*	10^8	Maltodextrin + water	Maltodextrin with sterile water	Nutrition–total calories delivered enterally, duration of hospital stay, death, oxygen therapy duration	0 (0)	No difference between probiotics and control group in all variables
Roy et al. (2014) (30)	112 (56/56)	DB; India	32 (2)/32.2 (2)	14/42; 16/40	1192 (341)/ 1069 (365)	Oral; 6 wk or until discharge	*L. acidophilis*, *B. longum*, *B. bifidum*, *B. lactis*	1.25×10^9, 1.25×10^8, 1.25×10^8, 1×10^9	Not mentioned	Breast milk	Full feed establishment, candida, death, NEC, hospitalization	0 (0)	Lower incidence in sepsis (*P* = 0.02) and hospitalization (0.002) in the probiotics group
Samanta et al. (2009) (49)	186 (91/95)	DB; India	30.12 (1.63)/30.14 (1.59)	Not mentioned	1172 (143)/ 1210 (143)	Oral; until discharge	*B. infantis*, *B. bifidum*, *B. longum*, *L. acidophilus*	2.5×10^9	Breast milk	Breast milk	NEC, death, hospitalization	0 (0)	Duration of hospital stay was lower (13.76 vs. 19.2, $P < 0.001$), lower NEC incidence (5.5% vs. 15.8%, *P* = 0.042) and lower incidence of sepsis (14.3% vs. 29.5%, *P* = 0.02) in the probiotics group
Sari et al. (2012) (28)	221 (110/111)	DB; Turkey	29.7 (2.5)/29.8 (2.3)	46/40; 49/62	1241 (264)/ 1278 (273)	Oral; 36 (22.5)/35.2 (24.9)	*L. sporogenes*	3.5×10^8	Breast milk and preterm formula	Breast milk or formula	Weight gain, length, HC, CLD, hospitalization, NEC, sepsis, feeding intolerance, oxygen days, full feeding days	47 (21.3)	No difference between probiotics and control group in all variables
Serce et al. (2013) (50)	208 (104/104)	DB; Turkey	28.7 (2.1)/28.8 (2.2)	50	1162 (216)/ 1126 (232)	Oral; until discharge	*S. boulardii*	Not mentioned	Breast milk and preterm formula	Breast milk with distilled water	NEC, weight gain, death, hospitalization, sepsis, time to reach 100 $mL \cdot kg^{-1} \cdot d^{-1}$ of oral feeding	36 (14.8)	No difference between probiotics and control group in all variables
Stratiki et al. (2007) (51)	75 (41/34)	SB; Greece	31.5 (2.5); 31(2.75)	23/18; 17/17	1420 (220)/ 1400 (50)	Oral; 30 d	*B. lactis*	2×10^7	Preterm formula	Formula	Weight, length, NEC, time to full enteral feed	4 (4.9)	No difference between probiotics and control group in all variables
Totsu et al. (2014) (20)	283 (153/130)	DB; Japan	28.6 (2.9)/28.5 (3.3)	87/66; 71/59	1016 (289)/998 (281)	Enteral; until body weight reached 2000 g	*B. bifidum*	2.5×10^9	Water, breast milk, and formula	500 mg dextrin	Body weight, HC, hospitalization, sepsis, accelerated establishment of enteral feeding, death, NEC, CLD	50 (17.7)	Sepsis rate is lower in probiotics group than control group (3.9% vs. 10.0%, $P < 0.05$)
Van Niekerk et al. (2014) (31)	184 (91/93)	DB; South Africa	Overall mean 28.7	43/48; 43/50	Not mentioned	Enteral; 28 d	*B. infantis*	$3.5\ 6 \times 10^9$	Breast milk	Breast milk with MCT oil	Daily weight gain, length, HC, feeding amount (day 14, 28)	28 (15.2)	Higher length score in probiotics group ($P < 0.01$)
Chowdhury et al. (2016) (52)	102 (52/50)	DB; Bangladesh	31.38 (0.93)/31.68 (0.84)	33/19; 36/14	1310.6 (110.41)/ 1338.0 (97.71)	Oral/oral	Capsule TS6 probiotic containing *Bifidobacterium* species, *Lactobacillus*	6×10^9	Breast milk	Breast milk	NEC, hospital stay	0	Lower NEC rate and shorter hospital stay

(*Continued*)

TABLE 1 (*Continued*)

Study (year) (ref.)	Participants at beginning, *n* (P/C)	Design; location	Gestational age, P/C, wk	Sex, *n* P/C, M/F	Birth weight, P/C, g	Probiotic route of administration; duration, P/C	Probiotics strain(s)	Dose, CFU/g	Form of probiotics	Placebo group	Measured outcomes and results	Attrition, *n* (%)	Key results
Manzoni et al. (2009) (53)	319 (151/168)	DB; Italy	29.8 (2.8)/29.5 (3.2)	72/79; 86/82	1138 (253)/ 1109 (269)	Oral/oral	Bovine lactoferrin + *L. rhamnosus* GG	6×10^9	Breast milk	5% glucose solution	Sepsis, NED, mortality, IVH	0	Lower NEC, sepsis, mortality, and IVH rates
Manzoni et al. (2014) (54)	496 (238/258)	DB; Italy and New Zealand	29.6 (2.8)/29.4 (3.1)	116/122; 134/124	1129 (242)/ 1118 (259)	Oral/oral	Lactoferrin + *L. rhamnosus* GG	6×10^9	Breast milk	Breast milk	NEC, mortality	0	Lower NEC and mortality rates

[1] Values are means (SDs) unless otherwise indicated. CLD, chronic lung disease; DB, double blinded; HC, head circumference; IVH, intraventricular hemorrhage; MCT, medium-chain TG; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; P/C, probiotic group/control group; PMA, postmenstrual age; RDS, respiratory distress syndrome; ref., reference; ROP, retinopathy of prematurity; SB, single blinded; TPN, total parenteral nutrition.

rate in the probiotics group was 3.6% compared with 6.9% in the control group. The meta-analysis showed there was a significantly lower risk ratio in NEC by 45.0% (95% CI: 0.43%, 0.70%; $P < 0.001$) in the probiotics group compared with the control groups. The heterogeneity was not statistically significant ($I^2 = 10.7\%$, $P = 0.31$). The forest plot of the effect is presented in **Figure 2**A (see also **Table 2**).

A subgroup analysis (**Table 3**) found that the effect of the probiotics on NEC was statistically significant when the study quality was high ($P < 0.001$) compared with studies of moderate quality ($P > 0.05$). It was also found that the effect of probiotics on NEC was statistically significant when it was in a dosage $<10^9$ CFU ($P < 0.001$) compared with those trials that used a larger dosage of $\geq 10^9$ CFU ($P > 0.05$). Probiotics were more effective when they consisted of multiple strains ($P < 0.001$) compared with a single strain ($P < 0.05$). The trials that used a combination of breast milk and preterm formula ($P < 0.001$) as a medium to administer probiotics had better results compared with trials that used other forms ($P > 0.05$). A significant effect was also found in trials with infants <29 wk of gestation ($P < 0.001$) compared with trials with infants >29 wk of gestation. The effect was not different between trials that used probiotics for <6 wk compared with trials of >6 wk.

Effects on sepsis. Twenty-four studies, with 7894 infant participants, measured rates of infant sepsis. The mean sepsis rate in the probiotics group was 15.1% compared with 18.5% in the control group following probiotics administration. The meta-analysis showed that there was a significantly lower risk ratio of 17% (95% CI: 0.71%, 0.96%; $P = 0.02$) in the probiotics group compared with the control group. The heterogeneity was significant ($I^2 = 48\%$, $P = 0.01$). The forest plot of the effect is presented in Figure 2B (see also Table 2).

Subgroup analysis (Table 3) indicated that the effect of probiotics on sepsis was statistically significant in reducing the incidence of sepsis when the study quality was moderate ($P < 0.05$) compared with high-quality studies ($P > 0.05$). It was also found that the effect of probiotics on sepsis was statistically significant in reducing its incidence when probiotics consumption was $<10^9$ CFU ($P < 0.05$) compared with $>10^9$ CFU ($P > 0.05$). A significant effect was also found in trials of infants with gestational age <29 wk ($P < 0.01$) compared with trials of infants with a gestational age of ≥29 wk ($P > 0.05$).

Effects on mortality. Twenty-one studies with 7332 participants measured infant mortality. The mean mortality rate in the probiotics group was 6.8% compared with 8.8% in the control group. The meta-analysis showed that there was a significantly lower risk ratio in mortality of 22% (95% CI: 0.66%, 0.93%; $P = 0.01$) in the probiotics group compared with the control group. The heterogeneity was not significant ($I^2 = 7\%$, $P = 0.37$). The forest plot of the effect is presented in Figure 2C (see also Table 2).

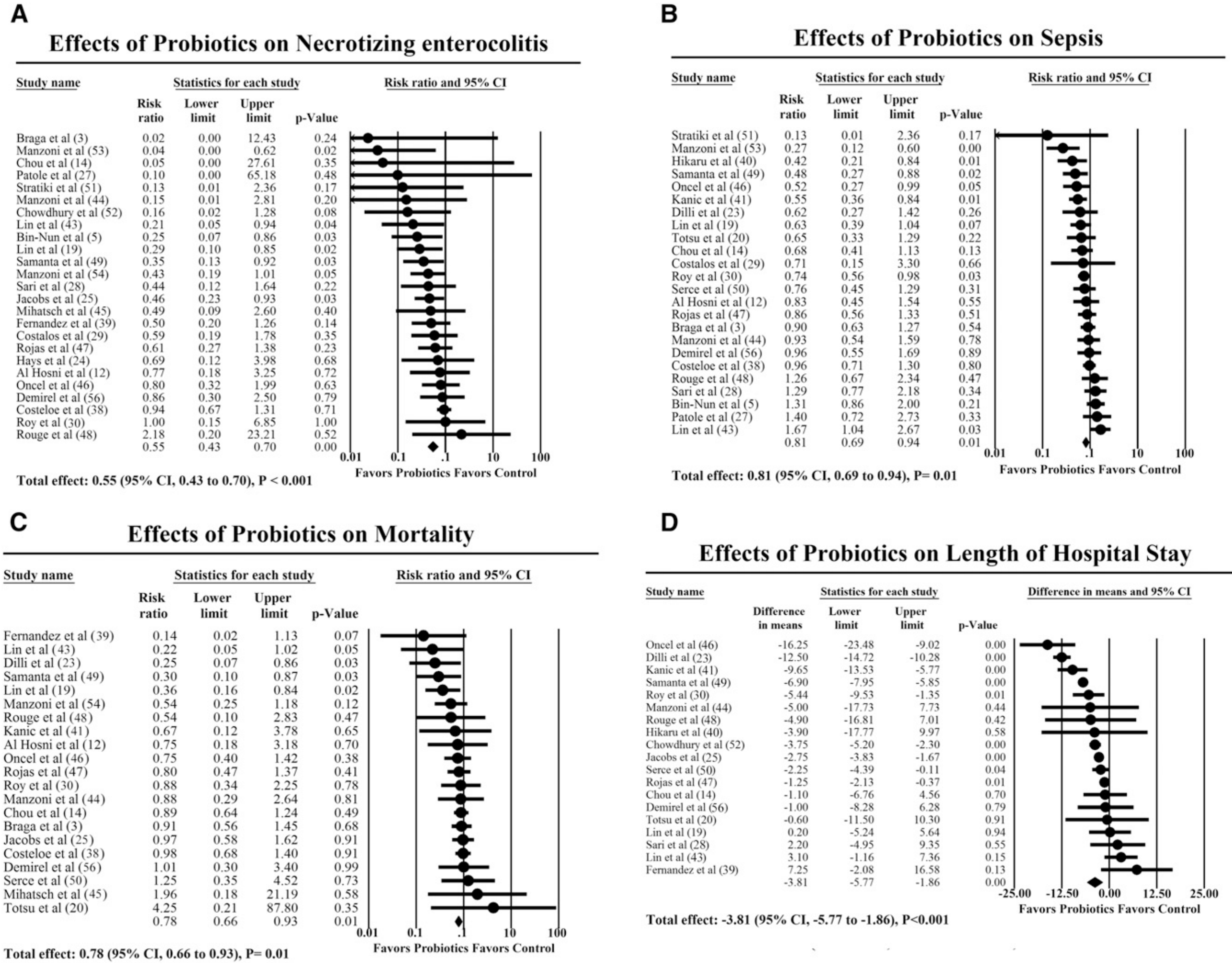

FIGURE 2 Forest plots of the effects of probiotics on necrotizing enterocolitis (A), sepsis (B), mortality (C), and length of hospital stay (D).

Subgroup analysis (**Table 4**) found that the effect of the probiotics on mortality was statistically significant when trials were of high quality ($P < 0.001$) compared with those trials of moderate quality ($P > 0.05$), when the dosage was $<10^9$ CFU ($P < 0.05$) compared with a larger dosage of $\geq 10^9$ CFU, when the probiotics consisted of multiple strains ($P < 0.001$) rather than a single strain, and when the trials used breast milk and formula ($P < 0.01$) compared with trials that used other forms ($P > 0.05$). A significant effect was also found in trials with infants of <29 wk gestation ($P < 0.05$) compared with trials with infants of >29 wk gestation and when the duration of probiotics administration was ≤6 wk ($P < 0.05$) compared with trials ≥6 wk ($P > 0.05$).

Effects on length of hospital stay. Nineteen studies with 5443 infant participants measured length of hospital stay. The mean hospital stay time in the probiotics group was 48.07 d (SD: 20.82 d) compared with 50.40 d (SD: 21.65 d) in the control group. The meta-analysis demonstrated a significantly reduced hospital stay by 3.81 d (95% CI: −5.77; −1.86 d; $P < 0.001$) in the probiotics group compared with the control group. The heterogeneity was

TABLE 2 Total effects of probiotics on necrotizing enterocolitis, sepsis, mortality, hospital stay, intraventricular hemorrhage, and weight gain

Variable	Studies, *n*	Risk ratio or mean difference	95% CI	*P*	I^2	I^2 *P*
Necrotizing enterocolitis, risk ratio	25	0.55	0.43, 0.70	<0.001	10.68	0.31
Sepsis, risk ratio	24	0.83	0.71, 0.96	0.02	48	0.01
Mortality, risk ratio	21	0.78	0.66, 0.93	0.01	6.92	0.37
Hospital stay, mean difference, d	19	−3.81	−5.77, −1.86	<0.001	89.62	<0.001
Intraventricular hemorrhage, risk ratio	10	0.91	0.73, 1.14	0.42	0	0.63
Weight gain, mean difference, g	15	−0.29	−1.16, 0.58	0.51	0	0.97

TABLE 3 Subgroup analysis on the effects of probiotics on necrotizing enterocolitis and sepsis[1]

	Necrotizing enterocolitis							Sepsis						
Subgroups	Studies, *n*	Participants, *n*	I^2	Q-test	Risk ratio	95% CI	*P*	Studies, *n*	Participants, *n*	I^2	Q-test	Risk ratio	95% CI	*P*
Quality of study							0.01							<0.02
High	15	3374	0.00	10.94	0.44***	0.33, 0.60		12	2357	68.53**	34.95	0.90	0.70, 1.16	
Moderate	10	981	0.00	8.44	0.79	0.60, 1.03		11	1101	14.36	12.85	0.78***	0.54, 1.12	
Dosage							0.42							0.57
$<10^9$	13	2410	0.00	7.49	0.40***	0.28, 0.58		12	2508	68.40***	34.81	0.68*	0.46, 0.99	
$\geq 10^9$	11	1945	23.37	13.05	0.57	0.37, 0.90		10	1846	11.92	10.22	0.86	0.68, 1.08	
Strains							<0.01							0.77
Single	11	1792	0.00	8.10	0.74*	0.56, 0.97		11	2116	37.09	15.89	0.79	0.60, 1.03	
Multiple	14	2563	0.00	10.13	0.37***	0.26, 0.54		12	1342	63.56***	30.19	0.74	0.52, 1.04	
Form of probiotics							0.14							0.84
Breast milk and formula	16	5791	0.00	13.06	0.44***	0.33, 0.60		13	3082	63.49***	32.88	0.75	0.54, 1.04	
Other	7	2431	17.33	7.26	0.67	0.42, 1.07		9	3281	37.78	12.86	0.79	0.58, 1.07	
Gestational age, wk							0.66							0.48
<29	9	2262	0.00	6.15	0.42***	0.28, 0.64		13	2179	28.51	16.79	0.70**	0.56, 0.88	
≥29	10	1684	0.00	8.81	0.47***	0.33, 0.69		9	1011	73.40***	22.55	0.77	0.43, 1.40	
Duration of probiotics, wk							0.15							0.61
≤6	14	2094	0.00	9.23	0.41***	0.28, 0.60		9	1559	66.81**	24.10	0.63	0.39, 1.01	
>6	11	2261	20.72	12.61	0.60***	0.42, 0.87		7	1746	62.56**	16.03	0.81	0.54, 1.22	

[1] If there were ≤2 studies in each subgroup, they were excluded. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

significant ($I^2 = 89\%$, $P < 0.001$). The forest plot of the effect is presented in Figure 2D (see also Table 2).

Subgroup analysis (Table 4) found that moderate-quality studies had a significantly shorter length of hospital stay of ≤6.32 d (95% CI: −10.97, −1.68 d; $P < 0.01$) compared with high-quality studies (−2.32 d; 95% CI: −4.62, 0.01 d; $P > 0.05$). Probiotics with dosages of $<10^9$ CFU had a significantly greater effect in reducing the hospital length of stay (−3.66 d; 95% CI: −6.25, −1.08 d; $P < 0.01$) compared with dosages $\leq 10^9$ CFU (−2.2 d; 95% CI: −8.0, 3.7 d). Probiotics consisting of multiple strains had a significantly greater effect in reducing the length of hospital stay (−6.14 d; 95% CI: −6.90, −5.30 d; $P < 0.001$) compared with a single strain (−3.00 d; 95% CI: −4.55, −1.46 d; $P > 0.05$). Infants with a gestational age of <29 wk had a shorter length of hospital stay (−3.82 d; 95% CI: −7.09, −0.56 d; $P < 0.05$) compared with infants with a gestational age of ≥29 wk (−3.66 d; 95% CI: −6.16, −1.15 d). Infants who had <6 wk of probiotics consumption had a significantly shorter length of hospital stay (−4.32 d; 95% CI: −7.23, −1.41 d; $P < 0.05$) compared with infants who had ≥6 wk of probiotics consumption (−3.25 d; 95% CI: −6.59, −0.8 d; $P > 0.05$).

Effects on weight gain. Fifteen studies with 3751 infant participants measured weight gain as an outcome variable. The mean weight gain in the probiotics group was 16.41 g/d compared with 16.57 g/d in the control group. The meta-analysis showed that there was a 0.29-g lower weight gain in the probiotics group (95% CI: −1.16, 0.58 g, $P = 0.51$) compared with the control group, but this difference was not statistically significant. The forest plot of the effect is presented in **Figure 3**A (see also Table 2). The heterogeneity was unmeasurable ($I^2 = 0\%$, $P = 0.97$).

Subgroup analysis (**Table 5**) found that VLBW infants in the probiotics group who took breast milk or preterm formula had a significantly greater weight gain (mean weight gain = 2.20 g; 95% CI: 0.08, 4.48 g; $P < 0.05$) compared with those infants who received probiotics in other forms (weight gain = −0.89 g; 95% CI: −3.97, 2.18 g; $P = 0.57$). Other variables, including study quality, dosage amounts, number of probiotic strains, form of probiotics, and gestational age, had no effect on the weight gain.

Effects on IVH. Ten studies, with 3431 infant participants, reported the rate of IVH. The mean IVH rate in the probiotics group was 7.77% compared with 8.49% in the control group. The meta-analysis showed that there was a lower risk ratio of 9% (95% CI: 0.73%, 1.14%; $P = 0.42$) in the probiotics group compared with the control group, but this finding was not statistically significant ($P = 0.42$). The heterogeneity was not significant ($I^2 = 0\%$, $P = 0.63$). The forest plot of the effect is presented in Figure 3B (see also Table 2).

Subgroup analysis (Table 5) found that study quality, dosage amounts, number of strains of probiotics, form of probiotics, infant gestational age, and duration of probiotics administration had no effect on the rate of IVH.

TABLE 4 Subgroup analyses on the effects of probiotics on mortality and hospital stay[1]

Subgroups	Mortality: Studies, n	Participants, n	I^2	Q-test	Risk ratio	95% CI	P	Hospital stay: Studies, n	Participants, n	I^2	Q-test	Mean difference, d	95% CI	P
Quality of study							0.03							0.13
High	11	3080	4.99	10.53	0.58***	0.42, 0.80		11	1769	90.56***	95.61	−2.32	−4.62, 0.01	
Moderate	10	834	0	5.81	0.89	0.74, 1.06		8	903	90.34***	72.06	−6.32**	−10.97, −1.68	
Dosage							0.96							0.10
$<10^9$	10	1685	16.84	10.82	0.75*	0.57, 0.97		8	1402	85.03***	53.45	−3.66**	−6.25, −1.08	
$\geq10^9$	10	2125	14.89	10.57	0.78	0.57, 1.08		8	1064	89.28***	74.63	−4.15*	−8.18, −0.13	
Strains							0.02							0.49
Single	11	1844	0.0	2.54	0.94	0.75, 1.18		11	1063	58.42**	24.05	−3.00***	−4.55, −1.46	
Multiple	10	2340	41.75	15.45	0.58***	0.41, 0.81		6	1507	91.84***	85.81	−6.14	−6.90, −5.39	
Form of probiotics							0.38							0.80
Breast milk and formula	14	4542	13.06	14.95	0.74**	0.59, 0.93		12	3692	89.09***	100.78	−4.12**	−7.13, −1.12	
Other	6	2660	0	4.94	0.86	0.66, 1.12		6	1525	92.89***	70.29	−3.29*	−6.48, −0.09	
Gestational age, wk							0.01							0.93
<29	11	2223	19.71	12.45	0.74*	0.57, 0.97		9	1397	93.85***	130.18	−3.82*	−7.09, −0.56	
≥29	9	1383	0.00	7.39	0.73	0.54, 0.99		10	1173	78.01***	40.93	−3.66	−6.16, −1.15	
Duration of probiotics, wk							0.32							0.07
≤6	10	1735	8.52	10.93	0.69*	0.53, 0.93		8	1081	94.90***	137.13	−4.32*	−7.23, −1.41***	
>6	9	2026	3.69	8.31	0.84	0.69, 1.03		10	1370	72.88***	33.19	−3.25	−6.59, −0.08	

[1] If ≤2 studies in each subgroup, they were excluded. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Publication bias. The results for all factors except NEC showed minimal asymmetry, suggesting minimal publication bias. A visual inspection of the funnel plots showed no clear evidence of publication bias with regard to effects on NEC, sepsis, mortality, and length of hospital stay (**Supplemental Figure 1**), weight gain, or IVH (**Supplemental Figure 2**). Findings from the Egger's test (NEC: $P = 0.001$; sepsis: $P = 0.19$; mortality: $P = 0.07$; length of hospital stay: $P = 0.88$; weight gain: $P = 0.54$; and IVH: $P = 0.56$) supported the finding that there was no publication bias, except for NEC. Applying the trim and fill method did not change the overall effects for NEC when 9 studies were excluded from the analysis (3, 5, 12, 14, 27, 29, 41, 47, 51). These tests suggest that there was no statistically significant evidence of publication bias for any of the assessed outcomes.

Discussion

The pooled effects based on the meta-analysis found that probiotics improved VLBW infants' health outcomes, including reducing the incidence of major medical complications, such as NEC and sepsis, and reducing mortality and length of hospital stay. However, the effects of probiotics on weight gain and IVH were not statistically significant. These findings are important. With worldwide advances in neonatology and modern neonatal intensive care units, the incidence of NEC has increased in VLBW infants (55). These risk factors need to be addressed, because reductions in the risk of NEC and sepsis can significantly reduce the high mortality rate associated with a very preterm birth.

Our results are consistent with previous published reviews regarding the effects of probiotics on reducing the risk of NEC (16) and sepsis (17), subsequent mortality (16) and shortened length of hospital stay (18). However, unlike our study, previous reviews focused either on preterm infants with a gestational age <37 wk (17, 21), rather than VLBW infants or on 1 outcome variable (17). None of the studies provided subgroup analyses on key variables. Our study included RCT trials focusing on VLBW infants with a birthweight <1500 g or gestational age <32 wk. A subgroup analysis was also conducted to demonstrate the effect of probiotics administration in VLBW infants with a gestational age of <29 wk, and therefore, the data pertaining to this high-risk group could be extracted from the included studies.

Effects of probiotics on NEC, sepsis, mortality, and length of hospital stay. Subgroup analyses revealed that the significant effects of probiotics on reducing the risk of NEC, sepsis, and mortality and reducing the length of hospital stay may be due to the effects of probiotics dosage, probiotic forms (i.e., breast milk, preterm formula, and formula), number of strains in the products, duration of probiotics intake, and preterm infants' gestational age. Most of the trials used breast milk and formula (3, 5, 12, 14, 23–27, 39, 40–44, 46, 51, 53, 54, 56). Probiotics in breast milk form and preterm formula had a significant effect in enhancing weight gain. Probiotics in the breast milk and

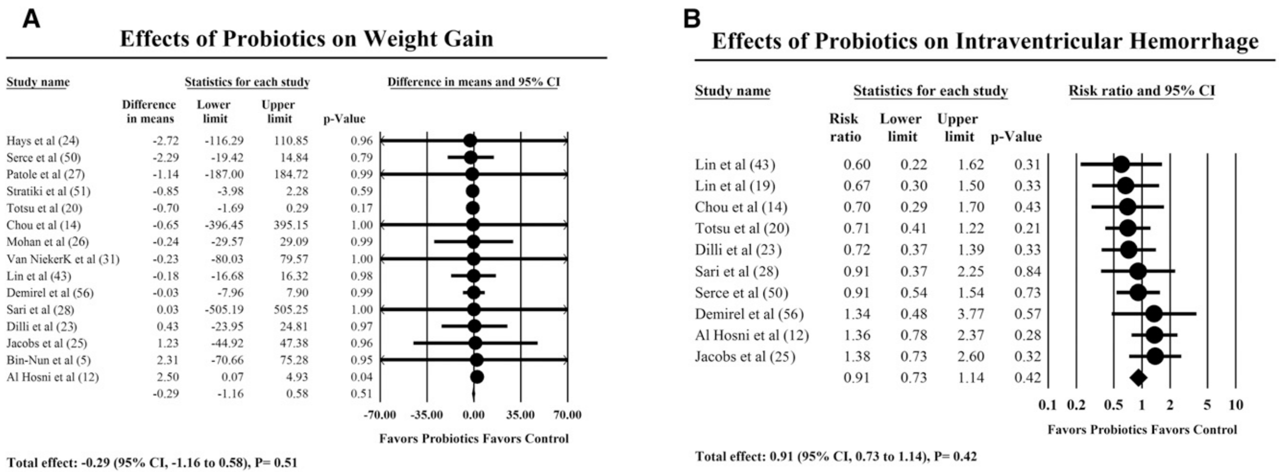

FIGURE 3 Effects of probiotics on weight gain (A) and intraventricular hemorrhage (B).

formula had significant pooled effects on the reduced risk of NEC and mortality, and probiotics in the other forms had no significant impact in reducing NEC and the risk of mortality. The heterogeneity of the studies was small, indicating the consistency of the findings in the effect of milk plus formula on the reduction of risk of medical complications and mortality and the effect of breast milk on enhancing weight gain.

The mechanism involved in the reduction of NEC during hospitalization may be related to the prevention of bacterial toxins and bacteria growth through "clearing" intestinal bacteria, the inhibition of potential pathogens to enhance immune response to the bacterial products' host, and the reduction in the incidence of intestinal infections (57). Use of human breast milk significantly reduces the risk of NEC and sepsis in the neonatal intensive care unit (58). Other potential mechanisms by which probiotics in breast milk may protect high-risk infants from developing NEC and mortality include increased barriers to migrating bacteria and their products across the mucosa (15), competitive exclusion of potential pathogens (15), modification of host response to microbial products (19), augmentation of IgA mucosal responses, and upregulation of immune responses (20). Another possible mechanism may occur because probiotics can reduce the degree of inflammation of the immature intestine of VLBW infants by entering the enteral feeding stage early and reducing hypoxia-related intestinal injury (59).

Fourteen trials that used multiple probiotic strains showed a statistically significant reduction of NEC and mortality and enhanced VLBW infant weight gain, whereas trials with a single strain did not. These results are similar to the finding that trials that used multiple probiotic strains had greater effects compared with those that used a single probiotics strain on the reduction of NEC and mortality and increase in body weight (4). In the studies we reviewed, a number of general bacteria were used in probiotics, including *Bifidobacterium infantis*, *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Bifidobacterium*, *Enterococcus*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Streptococcus*, and thermophiles. We found that *B. infantis* had the strongest effect on the reduction of risk of NEC, sepsis, and mortality. This may be because *B. infantis* can reduce diversity of the gut microbiota and pathogens, thereby optimizing its activity in the large intestine (60).

Probiotics in small dosages ($<10^9$ CFU) had a significant effect on the reduction of NEC, mortality, and length of hospital stay. The finding was confirmed in 13 studies for NEC and 8 studies for length of hospital stay. Probiotic supplementation in VLBW infants should be considered carefully to avoid overwhelming their immune system (1). Research recommends that VLBW infants be administered only 1.5×10^9 CFU/d of probiotics (61). This limitation is because infants in this population have impaired immune systems, poor nutrition, and frequent exposure to harmful microorganisms (1).

No significant differences were found on 2 variables, namely weight gain and rate of IVH. The lack of a significant difference in weight gain may be explained by measurement differences. In some studies, weight gain was measured daily (12), and in others, it was measured weekly (29) or when the study concluded, resulting in an averaging effect, which may mean that significant differences that occurred at particular times during the measurement period were missed. With regards to IVH, it should be noted that the number of VLBW infants with this condition included in the studies was insufficient to identify an effect. For example, there were only 6–8 infants in each of the studies cited (14, 19, 28, 42, 56) and a total of 279 with the IVH condition.

Strength and limitations. Our review used a thorough and comprehensive search strategy to identify and assess the quality of articles included in the meta-analysis. To minimize reviewer bias, authors conducted each step of this review independently before collaborating to decide on the included studies. A strength of our meta-analysis is that it included long-term intervention RCT studies with large sample sizes [with the exception of Mohan et al (26) and Stratiki et al (51)], so the power of the analyses was

TABLE 5 Subgroup analyses on the effects of probiotics on everyday weight gain and intraventricular hemorrhage[1]

	Everyday weight gain							Intraventricular hemorrhage						
Subgroups	**Studies, *n***	**Participants, *n***	**I^2**	***Q*-test**	**Mean difference, g**	**95% CI**	***P***	**Studies, *n***	**Participants, *n***	**I^2**	***Q*-test**	**Risk ratio**	**95% CI**	***P***
Quality of study							0.51							0.41
High	7	1051	0.0	0.68	2.17	−0.11, 4.45		5	934	0.00	1.66	0.86	0.60, 1.21	
Moderate	8	829	0.0	0.02	−0.71	−1.65, 0.23		5	789	22.15	5.14	0.95	0.69, 1.31	
Dosage							0.51							0.35
$<10^9$	5	816	0.0	2.75	1.24	−0.68, 3.15		5	965	15.02	4.71	1.00	0.70, 1.43	
$\geq10^9$	9	960	0.0	0.05	−0.69	−1.66, 0.29		5	758	0.00	1.50	0.83	0.62, 1.12	
Strains							0.51							0.42
Single	9	749	0.0	0.08	−0.71	−1.64, 0.23		5	578	0.00	2.93	0.96	0.71, 1.28	
Multiple	6	1131	0.0	0.11	2.44*	0.04, 4.83		5	1145	0.00	3.90	0.86	0.62, 1.20	
Form of probiotics							0.51							0.42
Breast milk and formula	10	3025	0.0	0.50	2.20*	−0.08, 4.48		8	2766	0.41	7.03	0.92	0.71, 1.17	
Other	3	459	0.0	0.03	−0.89	−3.97, 2.18		2	382	0	0	0.91	0.58, 1.44	
Birth weight, g							0.51							0.37
≥1000	12	1689	0.0	0.10	−0.70	−1.64, 0.23		9	1676	0.00	4.90	0.84	0.64, 1.10	
<1000														
Gestational age, wk							0.51							0.42
<29	8	1242	0.0	5.79	−0.25	−1.16, 0.66		7	1285	0.00	4.62	0.88	0.69, 1.13	
≥29	5	371	0.0	0.04	−0.73	−3.62, 2.16								
Duration of probiotics, wk							0.51							0.37
≤6	8	690	0.0	2.96	1.17	−0.72, 3.05		4	468	0.00	2.89	0.89	0.59, 1.32	
>6	6	1071	0.0	0.01	0.03	−7.73, 7.79		5	1102	0.00	2.93	0.99	0.67, 1.46	

[1] If ≤2 studies in each subgroup, they were excluded. *$P < 0.05$.

sufficient to generate firm conclusions. Any missing data were collected after contacting pertinent authors, and therefore all possible data were included in this meta-analysis at the time of publication. All studies involved RCT trials with VLBW infants, and therefore the effects of the probiotics could be assessed. A subgroup analysis to demonstrate the effect of probiotics administration in infants <29 wk of gestational age was performed, and therefore the data pertaining to this high-risk group could be extracted from the included studies. This meta-analysis builds on published findings. For example, a meta-analysis (16), which included 19 studies (5338 VLBW infants) reported no clinical effects of probiotics on sepsis, whereas our analysis had 24 RCT trials (n = 8492) with sufficient sample sizes that found probiotics reduced sepsis in VLBW infants.

A limitation of the present study is that there was inconsistent use of strain types among some of the studies, with some studies using one strain and others using multiple strains, making it difficult to assess which specific strain or strains contributed to the effect of the probiotics. Further RCTs involving long-term interventions that consistently compare specific single compared with multiple strains of probiotics supplement regimens are required to clarify these findings.

Clinical implications. The results of this study have identified that probiotics can provide a significant benefit to VLBW infant health, including a reduction of the incidence of major medical complications, such as NEC and sepsis, a reduction of mortality incidence, and a reduction in length of hospital stay. Considering these outcomes, the use of probiotics in neonatal intensive care units would be highly beneficial for VLBW infants to reduce the risk of developing medical complications, including NEC and sepsis. Through the provision of probiotics, taking into consideration appropriate dosage amounts, probiotic forms (i.e., breast milk, preterm formula, and formula), and multiple strains, VLBW infants would have the opportunity to strengthen their immune systems and optimize the amount of microbial activity in their large intestines. Finally, the results of this study highlight that probiotics provide positive outcomes for improving VLBW infants' health outcomes and that gestational age is also an important consideration. However, due to the multifaceted nature of probiotics, it is recommended that the process of probiotics intake become integrated into VLBW infants' usual care, providing them with ongoing monitoring and support.

In conclusion, the findings of this study suggest that supplementation with probiotics is effective in reducing the risk of major medical complications and the coexisting risk of mortality and shortens the length of hospital stay. Probiotics are more effective when taken in breast milk or breast milk plus formula form, consumed for <6 wk, administered at a dosage of $<10^9$ CFU/d, and include multiple strains. By managing the risks of medical complications and coexisting risk factors and through facilitating growth and enteral feeding, probiotics may provide an avenue to reduce the current high rates of mortality associated with NEC, sepsis, and delayed growth in VLBW infants.

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